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Using essential oils to control diseases in strawberries and peaches

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ABSTRACT

Strawberry and peach crops are of great economic and social importance, mainly due to the added value and income generation for small and medium producers in different regions of Brazil. Some fungal diseases can compromise the final profitability of production, such as those caused by *Colletotrichum* sp., *Botrytis cinerea* and *Monilinia fructicola* fungi. The control of these pathogens mainly occurs through fungicides, which has been generating concern for consumers, as well as biological imbalance and environmental contamination. The need for new alternatives for disease control has been leading to more research being conducted on essential oils. Our scientific questions were based on a compilation of experiments which revealed the efficiency of essential oils in disease control. With the purpose of evaluating the fungicidal activity of *Aloysia citriodora*, *Cymbopogon winterianus*, *Lippia alba* and *Ocimum americanum* essential oils on the control of fungi, such as *Colletotrichum* sp., *Botrytis cinerea* and *Monilinia fructicola in vitro* and in the post-harvest of fruits, this work was developed at the Federal University of Santa Maria, Frederico Westphalen county (Rio Grande do Sul state), Brazil, from 2016 to 2018. The following evaluations were done: (i) characterization of essential oil doses *in vitro* for controlling *Colletotrichum* sp., *Botrytis cinerea* and *Monilinia fructicola* fungi, and (ii) determination of the minimum inhibitory concentration (Ω, mL/L) of essential oils in post-harvest of strawberries and peaches. All essential oils have high fungicidal activity *in vitro* experiments. The *A. citriodora*, *L. alba* and *O. americanum* essential oils had a satisfactory effect for post-harvest controlling of *Colletotrichum* sp. *C. winterianus* and *O. americanum*. The essential oils promoted satisfactory post-harvest control of *Botrytis cinerea* in strawberries. All essential oils have high fungitoxicity against *Monilinia fructicola in vitro* and post-harvest, highlighting the greater efficiency of *A. citriodora* essential oil in peaches. The essential oils present high fungitoxicity for controlling diseases in strawberries and peaches, presenting high potential performance for formulating commercial fungicide.

1. Introduction

The *Rosacea* family includes important fruits such as strawberries and peaches. There was record strawberry production in Brazil during 2018 with 3481 tons, while 220 thousand tons of peaches and nectarines were produced (Faostat, 2020). In addition to the economic issue, cultivation has notable importance in the social context as it is an activity linked to family farming, in addition to generating employment and income (Camargo Filho and Camargo, 2009). However, the high perishability of these fruits is often related to the incidence of rot which directly affects the commercial product, causing qualitative and quantitative damage (Lopes et al., 2010), and reducing the final productivity.

Among the main diseases which promote losses in field production and in the post-harvest period of strawberries, anthracnose, caused by the *Colletotrichum* sp. fungus, and gray mold, caused by *Botrytis cinerea*, stand out. Regarding the peach culture, brown rot caused by *Monilinia fructicola* is one of the most destructive diseases in stone fruits (Hu et al., 2011).

The control measures for these diseases are mainly based on chemical fungicide sprays in the pre-harvest, and associated with the great sensitivity of the fruits and their importance makes them intense. The increase in cases of resistance to different mechanisms of action has already been observed for fungi such as *Botrytis*, *Colletotrichum* and *Monilinia* (Luo et al., 2008; Myresiotis et al., 2007; Primiano et al., 2017; Sang et al., 2018; Williamson et al., 2007).

The use of unauthorized active ingredients for the crop and the presence of residues above the maximum limits have made fruits the targets of the pesticide residue analysis program in food (Dos Estados,

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2013), generating concern for consumers. In light of this, the consumer market's frequent search for higher quality fruits has intensified the need for products that replace polluting inputs, increasing consumer safety and health (Piati et al., 2011).

In the search for new products with potential biological activity, much effort has been devoted to extracting and identifying natural products and secondary metabolites produced by plants traditionally used for their various healing abilities (Robles-Kelly et al., 2017). Several unconventional methods are being investigated with encouraging results, and essential oils are one of the most studied lines today (Aguilar-González et al., 2015), among others.

In addition to being highly effective, essential oils have become an alternative to satisfy consumer demands to purchase food products with less additives or natural treatments (Trajano et al., 2010). In this sense, essential oils are alternatives to increase the shelf life of fruits due to their antimicrobial activity and low risk for developing resistance to pathogens resulting from their complex composition and their different mechanisms of action (Rehman et al., 2016).

Several studies have been conducted to evaluate the effect of essential oils on the control of phytopathogenic fungi. It has been proven that essential oils from *Syzygium aromaticum* (cloves) and *Brassica nigra* (mustard) (Aguilar-González et al., 2015) can inhibit or delay the growth of *Botrytis cinerea* in strawberries (Sangsuwan et al., 2016). Anaruma et al. (2010) found activity of 15 essential oils against *Colletotrichum gloeosporioides*. *Lippia sidoides* essential oil reduces the development of anthracnose in both *in vitro* and post-harvest trials of strawberries (Oliveira et al., 2019a,b). Essential oils from plants such as *Eucalyptus globulus*, *Cinnamomum camphora* and *Cymbopogon citratus* have also been shown to be effective in controlling *Monilinia fructicola* (Pansera et al., 2015).

The essential oils are classified by the Food and Drug Administration (FDA) as safe for use in food, with increasing interest in using them for treating fruits and vegetables (González-Aguilar et al., 2008), and in their post-harvest (Feng et al., 2008). Thus, the search for new alternatives to preserve food which are mainly efficient, profitable and easy to obtain is a growing research field and is of great interest to the food industry (Aguilar-González et al., 2015).

Although some studies have proven the fungitoxicity of essential oils, herein we have sought to elucidate the behavior of *Aloysia citriodora*, *Cymbopogon winterianus*, *Lippia alba* and *Ocimum americanum* essential oils in the control of *Colletotrichum* sp., *Botrytis cinerea* and *Monilinia fructicola* fungi. The objective of the work was to evaluate the fungitoxicity of essential oils in the control of diseases in strawberry and peach fruits.

2. Materials and methods

2.1. Extraction of essential oils and analysis of chemical composition

Aloysia citriodora, *Cymbopogon winterianus*, *Lippia alba* and *Ocimum americanum* essential oils were extracted from fresh leaves of aromatic plants grown in the field, at the Federal University of Santa Maria, Frederico Westphalen campus, Rio Grande do Sul state, Brazil.

The essential oil extraction was performed by hydrodistillation using Clevenger devices and a chromatographic analysis was subsequently carried out to understand the chemical composition. The materials for analysis were packaged in amber bottles and sent to the Laboratory of Vegetable Extracts (LABEV), belonging to the Federal University of Santa Maria, in the municipality of Santa Maria. The analysis was performed by a CG-MS TIC gas chromatograph with a flame ionization detector. The essential oil constituents were identified based on the retention index (RI) and mass spectrum fragmentation models through the Agilent ChemStation program and the NIST database (Burgess et al., 2016), with the result expressed as a percentage.

2.2. Origin of isolates

Colletotrichum sp. was collected from symptomatic fruits identified under an optical microscope and the fungal structures were inoculated in Petri dishes (90 × 25 mm, J. Prolab®) containing PDA culture medium (Potato-Dextrose-Agar), and incubated in a BOD chamber for seven days at a temperature of 25 °C and a photoperiod of 12 h, followed by multiplication to carry out the experiments.

Botrytis cinerea isolate was obtained from symptomatic fruits collected in the UFSM experimental field. Fungal structures were transferred to Petri dishes containing SDA culture medium (Strawberry-Dextrose-Agar), and kept in a BOD chamber at a temperature of 23 °C under a photoperiod of 12 h for multiplication of the fungus and subsequent multiplication in the experiments.

The *Monilinia fructicola* isolate was collected from symptomatic individuals, identified under an optical microscope and the fungal structures were inoculated in Petri dishes containing PDA culture medium, incubated in a BOD chamber for 5 days at 25 °C and a 12-hour photoperiod, followed by multiplication to perform the experiments.

2.3. Mycelial growth

Two separate experiments for *Colletotrichum* sp. and *Botrytis cinerea* were carried out in a factorial scheme in a completely randomized design of 4 × 8 constituted by four essential oils (*Aloysia citriodora*, *Cymbopogon winterianus*, *Lippia alba* and *Ocimum americanum*) with eight doses of each oil, being 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 (mL of essential oil per liter of PDA). All treatments consisted of 10 replications, and the experimental unit was considered a Petri dish.

An experiment for *Monilinia fructicola* was carried out in a factorial scheme in a completely randomized design of 3 × 8 in which three essential oils (*Aloysia citriodora*, *Cymbopogon winterianus* and *Ocimum americanum*) and the eight doses of each oil were composed by 0.0, 0.2, 0.4, 0.6, 0.8, 1.0; 1.2 and 1.4 mL/L. Moreover, five doses were tested for the *Lippia alba* essential oil, being 0.0, 0.4, 0.8, 1.2 and 1.6 mL/L. All treatments consisted of 10 replications, with a Petri dish being considered as an experimental unit.

The treatments were incorporated into the PDA (potato-dextrose-agar) culture medium in a laminar flow chamber at 50 °C, together with the Tween 20 surfactant (0.05 mL), while only the PDA culture medium was used for the control (0.0 mL/L). After solidification of the media, mycelium fungi with 7.0 mm in diameter were transferred to the center of the Petri dishes containing their respective treatments. The inoculated plates were sealed with parafilm so that there was no evaporation of the constituents and then incubated in a BOD chamber at a temperature of 25 °C and a photoperiod of 12 h.

The diameter of the colonies related to the treatments was performed daily in an orthogonal position until the control reached the total diameter of the Petri dish in order to determine the Mycelial Growth Speed Index (μ) using the equation adapted by Oliveira (1991):

$$\mu = \frac{\sum_{i=1}^{n}(D_i - D_{i-1})}{n} \quad (1)$$

where D_i is the current average diameter, D_{i-1} is the average diameter of the previous day, and n is the number of days after setting up the experiment.

The last measurement was performed (mean of two measures of opposite diameters) on the 10th day of evaluation for the *Monilinia fructicola* and *Colletotrichum* sp. fungi, and on the 5th day for *Botrytis cinerea*, therefore being able to determine the Mycelial Growth (MG). From the average treatment values with the dose of 0.0 mL/L (control), it was possible to evaluate the variable growth inhibition percentage (GIP), which was then calculated for each concentration in relation to the control values (Balbi-Peña et al., 2006). The minimum inhibitory

concentration (Ω, mL/L) of the fungi was determined according to the results to facilitate visualization. Ω represents the minimum dose of essential oil to inhibit the fungus growth.

The inhibition percentage values (GIP) of treatments were used to determine the EC_{50} by adjusting the linear regression, which corresponds to the concentration C of the product necessary to control 50% ($GIP = 50\%$) of the fungus:

$$GIP = a \cdot C + b \quad (2)$$

From the plotted linear regression, the following formula was used for obtaining the effective concentration of 50%:

$$EC_{50} = \frac{50 - b}{a} \quad (3)$$

2.4. Spore germination for Monilinia fructicola

To determine the effect of essential oils on spore germination, the experiment was conducted in a factorial scheme in a completely randomized design (CRD) of 4 × 7 with the four essential oils mentioned above and seven doses, being (0.0, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mL/L). All treatments consisted of eight replications, with an Elisa plate cavity.

A 1.0 mL aliquot of the spore suspension of *Monilinia fructicola* containing 1.08×10^4 spores was mixed in the 4.0 mL solution of autoclaved distilled water according to the concentrations of essential oils, totaling 2.17×10^3 spores/mL. Next, 300 μL of this solution was used to place in each of the Elisa plate containers (Regente et al., 1997). The plates were incubated in a BOD chamber at 25 °C and a photoperiod of 12 h, with the germination of conidia determined 24 h after beginning the experiment (Kososki et al., 2001). Next, 20 μL of the lactophenol blue dye was used for stopping spore germination. The evaluation was performed by observing a 100 μL aliquot of the solution under an optical microscope using a hemocytometer.

Germinated conidia were considered to be those which had the length of the germ tube greater than or equal to the diameter of the conidium (Silva et al., 2009). A total of 100 spores were counted per repetition, and the percentage of germinated spores was estimated from the total number of germinated and non-germinated conidia.

2.5. Post-harvest

2.5.1. Strawberries

The best results for minimum inhibitory concentration (Ω, mL/L) of the mycelial growth post-harvest experiment (*in vitro*) were analyzed in strawberries, Albion cultivar, for evaluating the efficiency of essential oils to control *Colletotrichum* sp. and *Botrytis cinerea* fungi. The ripe fruits were purchased in the Frederico Westphalen city (Rio Grande do Sul state), being selected at random, and free from contaminants or injuries. The fruits were superficially disinfected by immersion for 1 min in a sequence of solutions (70% alcohol, 1% sodium hypochlorite, and washed in sterile water).

The experimental design used for treating the strawberries in the post-harvest was completely randomized in a 5 × 2 factorial scheme with five essential oils, being Control, *Aloysia citriodora*, *Cymbopogon winterianus*, *Lippia alba* and *Ocimum americanum*. There were two forms of inoculation constituted by artificial inoculation and without artificial inoculation, totaling 10 treatments and 16 replications per treatment were used, with each fruit being considered an experimental unit.

The essential oils were mixed with 1 l of water (maintaining the indicated concentrations), and the Tween 20 surfactant (0.05 mL) was used. The fruits were immersed for 1 min in this solution. After immersing the fruits in the treatments, they were naturally dried under sterile paper and then the treatments with artificial inoculation were carried out with a 10 μL aliquot of a suspension with 7.9×10^4 spores/mL of *Colletotrichum* sp., and 6.56×10^3 spores/mL of *Botrytis cinerea* by an injection with a sterile syringe in the equatorial region of the fruit. The fruits without artificial inoculation did not receive inoculum or injury, representing the possible latent infections which may be present in the fruits.

The fruits were put in 36 × 20 cm^2 plastic trays with transparent cover on plastic rings made from straws, placed under paper towels, moistened with distilled water to maintain humidity and stored on a bench with a controlled temperature of 25 °C for *Colletotrichum* sp. and 23 °C for the experiment with *Botrytis cinerea*, for 72 h.

On the last day of evaluation, the incidence, severity, control efficiency, diameter of the lesion and spore production variables were analyzed. The incidence was analyzed based on the presence or absence of typical symptoms of the disease and the severity through the percentage of the fruit affected by the disease, and the results were expressed in percentage. The lesion diameter variable was based on the average length and width of the lesion in orthogonal directions, and the results were expressed in centimeters. The analysis of the treatment control efficiency was performed from the values of the diameter of the lesion in relation to the control, expressed in percentage.

Spore production was estimated by washing the fruits with a soft bristle brush in 10 mL aliquots of distilled water and 0.05% Tween. The aliquots were shaken in test tubes in a vortex apparatus and stored in Eppendorf for later reading. Spore production was analyzed using 100 μL per hemocytometer sample and readings performed under an optical microscope. Four readings were performed per replication and the results were expressed in spore production per mL.

2.5.2. Peaches

In order to evaluate the efficiency of essential oils for controlling *Monilinia fructicola* (*in vivo*) in peach, Precocinho cultivar, the fruits were taken to the Phytopathology laboratory at UFSM, and superficial disinfestation was carried out following the same methodology previously mentioned.

The experimental design used for the treatment of fruits in the post-harvest was completely randomized in a 5 × 2 factorial scheme with five treatments, being Control, *Aloysia citriodora*, *Cymbopogon winterianus*, *Lippia alba* and *Ocimum americanum*. There were two forms of inoculation, constituted with artificial inoculation and without artificial inoculation, totaling 10 treatments, and 10 replications per treatment were used, with each fruit being considered an experimental unit.

The essential oils were mixed with 1 l of water (maintaining the indicated concentrations), and the Tween 20 surfactant (0.05 mL) was used. The fruits were immersed for 1 min in this treatment solution. After immersing the fruits, they were dried on sterilized paper and the treatments with artificial inoculation were inoculated with mycelium discs (7 mm) of *Monilinia fructicola* by injuring the equatorial region of the fruit with the aid of a pourer. Fruits without artificial inoculation only received post-harvest treatments with essential oils in order to verify the influence of latent infections. The fruits were put in 36 × 20 cm^2 plastic trays with a transparent lid with 50 mm diameter and 2 cm high plastic polyvinyl chloride (PVC) rings, placed on a paper towel moistened with distilled water to maintain humidity, and kept on a bench with controlled temperature at 25 °C for 96 h.

The evaluations started 24 h after immersion in the treatments and were carried out daily until 96 h of incubation. The variables of incidence, severity, lesion diameter, control efficiency and spore production were analyzed on the last day of evaluation. The infection severity of fruits by *Monilinia fructicola* was evaluated daily, based on the scale of Wagner Júnior et al. (2005), with scores from 0 to 4: [score 0] fruit without infection, [score 1] from 0 to 25% of the fruit surface with disease damage, [score 2] from 25 to 50% of the fruit surface with damage to the disease, [score 3] from 50 to 75% of the fruit surface with disease damage, and [score 4] greater than 75% of the fruit surface with disease damage.

Based on the severity scores, the area under the disease progress severity curve (α, % day) was determined using the following formula:

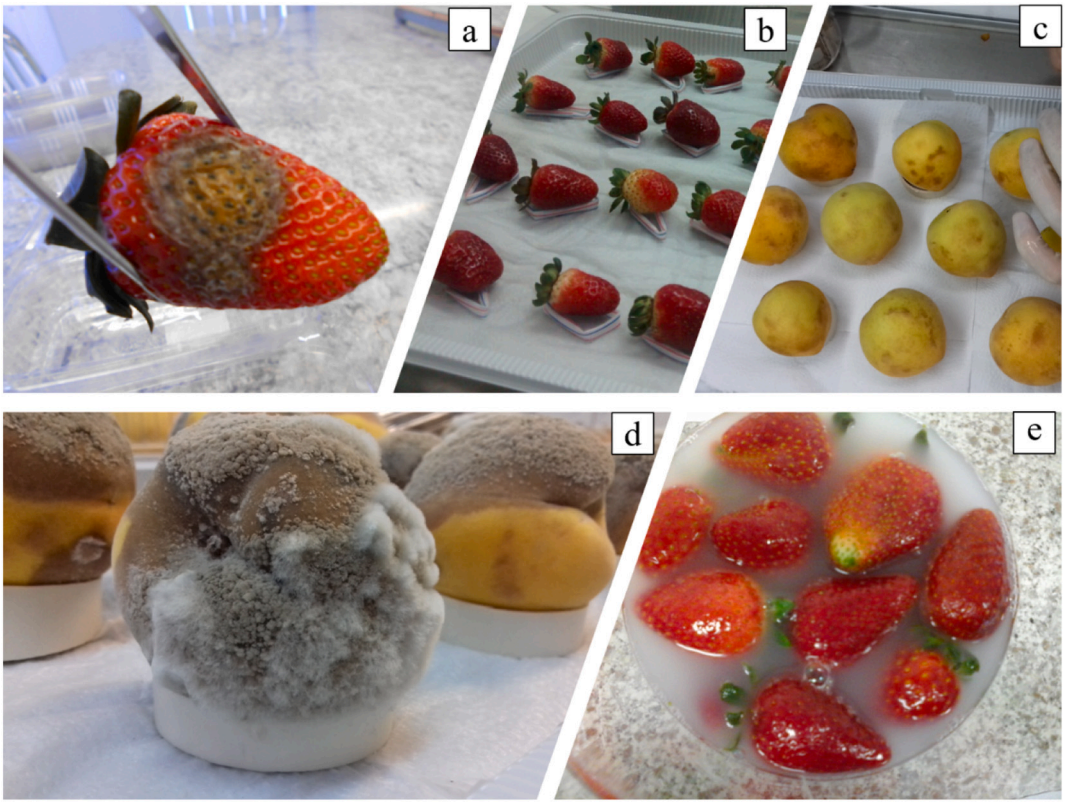


Fig. 1. Symptoms of gray mold and brown rot in strawberry (a) and peach (d) fruits, artificial inoculation with pathogens (b, c) and post-harvest treatment with immersion (e). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

$$\alpha = \frac{\sum_{i=1}^{m}(I_i + I_{i+1})(t_{i+1} - t_i)}{2} \quad (4)$$

where I_i is the severity (%) at the time i of assessment, and t_i is the number of days after post-harvest treatment. An overview of the experiment can be seen in Fig. 1.

2.6. Statistical analysis

For the *in vitro* experiment, the variables μ, MG and GIP were subjected to analysis of variance and a means test was performed when significant.

For post-harvest, the variables of incidence (IN, %), severity (S, %), lesion diameter (LD, cm), area under the disease progress severity curve (α) (only for *Monilinia fructicola*), control efficiency and spore production were analyzed by analysis of variance, while the means of the treatments were compared by the Tukey test at 5% of probability when significant with the R statistical program (Version 3.0) using the *ExpDes.pt* and *Laercio* packages.

3. Results

3.1. Analysis of the chemical composition of essential oils

The *Aloysia citriodora* essential oil presented β-citral (27.66%), cis-citral (20.68%) and limonene (19%) as major compounds. The *Cymbopogon winterianus* oil showed cis-geraniol (32.85%), β-linalool (29.33%) and citronellal (14.50%) in its composition. The *Lippia alba* oil presented 66.57% of β-linalool as a major compound, while the *Ocimum americanum* essential oil presented cineole (35.71%), alcanfor (12.50%), p-eugenol (11.42%) and β-linalool (11.26%) as major compounds (Table 1).

3.2. Mycelial growth

According to the analysis of variance, it was possible to verify a significant difference for the doses of essential oils in the analyzed variables. All essential oils were fungitoxic for the mycelial growth of *Colletotrichum* sp., *Botrytis cinerea* and *Monilinia fructicola*, reducing their fungal growth as the dosages increased (Table 2).

The minimum inhibitory concentration (Ω, mL/L) of each essential oil used to control fungi was used for post-harvest tests, and can also be seen in Table 2.

The inhibitory dose of the *C. gloesporioides* mycelial growth was 1.2 mL/L for all essential oils. Mycelial growth was reduced more sharply when submitted to *A. citriodora* oil when compared to the others, presenting the lowest EC_{50} value, with only 0.41 mL/L. The highest EC_{50} values were observed for the C. *winterianus* essential oil, with 0.86 mL/L (Table 2).

The *A. citriodora* essential oil showed greater antifungal potential in the control of *Botrytis cinerea*, as it required low doses to inhibit the growth of the fungus, with 0.8 mL/L. The *C. winterianus* and *L. alba* essential oils inhibited the development of the fungus at a concentration of 1.0 mL/L. The fungitoxicity of the *O. americanum* essential oil was low for this fungus, inhibiting growth at a dose of 1.4 mL/L. The lowest EC_{50} was found for the *C. winterianus* essential oil, with 0.52 mL/L, while the highest values were observed for *O. americanum* oil, with 0.74 mL/L (Table 2).

The *A. citriodora* and *C. winterianus* essential oils showed high fungitoxicity for *Monilinia fructicola*, with reduced mycelial growth as the essential oil dose increased, and inhibition in the dose of 0.4 mL/L for both. These essential oils had the lowest EC_{50} values, with 0.21 and 0.23 mL/L, respectively. On the other hand, the *L. alba* and *O. americanum* essential oils showed less fungitoxicity against *Monilinia fructicola* when compared to other oils, presenting mycelial growth inhibition at doses of 1.2 and 0.8 mL/L, respectively. The lower fungitoxicity can be observed

Table 1
Chemical composition (%) of the *Aloysia citriodora*, *Cymbopogon winterianus*, *Lippia alba* and *Ocimum americanum* essential oils.

Chemical component	Essential oils			
	A. citriodora	*C. winterianus*	*L. alba*	*O. americanum*
Alloaromadendrene	–	–	4.11	–
Alcanfor	–	–	–	12.50
Limonene	19	–	–	–
α-Limonene	–	1.00	–	3.84
β-Cis-ocimene	0.46	–	–	–
β-Linalool	0.17	29.33	66.57	11.25
Myrtenol	0.49	–	–	–
2-Pinen-10-ol	1.17	–	1.95	–
Cis-citral	20.68	–	–	–
Trans-citral	–	0.50	–	–
Germacrene b	–	3.08	–	–
Trans-geraniol	0.34	–	–	–
Cis-geraniol	–	32.85	2.1	–
β-Elemene	–	0.63	–	–
β-Citral	27.66	–	1.6	–
Citronellal	–	14.50	–	–
P-cymene	–	–	1.190	–
Citronellol acetate	–	2.17	–	–
Citral b	–	1.12	–	–
α-Cubebene	–	–	0.40	–
Geraniol acetate	2.10	–	–	–
Cineole	–	–	3.3	35.71
Caryophyllene	7.32	–	4.3	–
ß-Caryophyllen	–	–	–	0.87
α-Curcumene	5.47	–	–	–
Cedreanol	–	0.83	–	–
Cis-α-bisabolene	1.88	–	–	–
Cyclohexane	–	–	0.53	–
Zingiberene	0.86	–	–	–
δ-Cadinene	0.11	3.26	–	–
P-eugenol	–	–	–	11.42
α-Terpinol	–	–	–	6.45
Spathulenol	2.46	–	–	–
Caryophyllene oxide	5.78	–	–	–
Santolina triene	–	–	0.31	–
Naphthalene	–	–	0.60	–
Germacrene d	–	2.54	6.70	5.11
Terpinene	–	–	–	0.40
2-Norpinene	–	–	–	0.68
δ-Cadinol	1.07	4.59	–	–
Total identified	96.65	96.40	93.73	88.00

in the EC_{50} values as well, with 0.41 mL/L for both essential oils (Table 2).

3.3. Spore germination

According to the analysis of variance performed, the factors essential oils x oil doses showed significance for the variable of spore germination percentage (Table 3).

The *A. citriodora* essential oil reduced the germination of the spores as the dose increased, promoting inhibition of the germination percentage in the concentration of 0.2 mL/L. For the *C. winterianus* essential oil, the dose which promoted germination inhibition of *M. fructicola* spores was 0.4 mL/L. On the other hand, the *L. alba* and *O. americanum*, essential oils reduced spore germination as the tested dosage increased, however there was no spore germination inhibition at the highest dose with 1.2 mL/L of the essential oil (Table 3).

3.4. Post-harvest

3.4.1. Colletotrichum sp.

According to the analysis of variance performed, the variables: incidence, spore production and control efficiency for *Colletotrichum* sp. showed significance for the interaction between the essential oils x forms of inoculation. However, the lesion diameter and severity variables were only significant for the essential oils factor.

The inoculation forms showed significant differences for the tested essential oils and artificially inoculated strawberries, regardless of the treatment of essential oils, had a higher incidence and spore production than uninoculated fruits (Table 4, Supplementary Fig. 1).

The *A. citriodora* essential oil stood out from the others for inhibiting fungal development, not promoting the disease incidence, or the production of spores for both strawberries with artificial inoculation and for fruits without inoculation, demonstrating 100% control efficiency. The *O. americanum* essential oil also stood out for its high control efficiency for fruits which were not artificially inoculated, with 80% control efficiency (Table 4).

The *L. alba* essential oil significantly reduced the incidence and spore production of *Colletotrichum gloesporioides* in strawberries with artificial inoculation, promoting 47% of control efficiency (Table 4).

The *A. citriodora* essential oil again stood out for inhibiting the severity and diameter of the lesion of *Colletotrichum* sp. The *C. winterianus* essential oil was not very efficient, as it allowed the disease to develop, as can be seen in injury severity and diameter values. Although the other essential oils showed low values, they were statistically similar to the control (Table 4).

3.4.2. Botrytis cinerea

According to the analysis of variance performed, all variables were significant for the interaction between the essential oils x forms of *Botrytis cinerea* inoculation. All variables generally showed higher levels of disease in artificially inoculated strawberries, however the presence of the disease was verified in non-artificially inoculated fruits, thus ensuring the importance of observing the latent effect of fungi.

The statistical difference for the inoculation form was more pronounced for the variable incidence of *Botrytis cinerea*, as strawberries with artificial inoculation showed a higher incidence of the disease. Treatments for fruits without artificial inoculation did not influence the disease incidence. The highest disease values were observed when strawberries were inoculated; however, there was a similarity between treatments for the other variables when analyzing the inoculation factor.

The *C. winterianus* essential oil stood out from the others, promoting a low incidence of the disease in inoculated strawberries with approximately 30%. This essential oil promoted satisfactory control of the disease, as seen in the other variables, such as reduced severity, spore production, lesion diameter and reaching 100% control in fruits without inoculum, and 89% control in inoculated fruits (Table 5, Supplementary Fig. 2).

The treatment with *O. americanum* essential oil provided similar results to the *C. winterianus* essential oil, showing statistical equality, and therefore efficient control, with values of approximately 100% control for peaches without inoculation and 85% of fruits with inoculation. The *A. citriodora*, *C. winterianus* and *O. americanum* essential oils showed excellent performance as post-harvest treatments on peaches without artificial inoculation, completely inhibiting fungal development and achieving 100% control efficiency (Table 5).

However, a high incidence of the disease promoted by *Botrytis cinerea* was observed in fruits treated with the *L. alba* essential oil, with 100% for fruits with inoculation and 25% for fruits without artificial inoculation, and not statistically differing from the control. In this same sense, the *A. citriodora* essential oil also demonstrated low potential for controlling *Botrytis cinerea* in inoculated fruits (Table 5).

3.4.3. Monilinia fructicola

The analysis of variance showed significance in the interaction between the essential oils x forms of inoculation for the variables of incidence, severity, lesion diameter, spore production and control efficiency. The area under the disease progress severity curve (α) for *Monilinia fructicola* was only significant for the essential oils and inoculum factors. The inoculation process was indifferent in the quantification of all the variables analyzed for the control (Table 6, Supplementary Fig. 3).

Table 2
Mycelial growth (MG), inhibition control percentage (ICP), mycelial growth speed index (μ), minimum inhibitory concentration (Ω, mL/L) and EC_{50} for the *Colletotrichum gloesporioides*, *Botrytis cinerea* and *Monilinia fructicola* fungi when submitted to doses (D, mL/L) of *Aloysia citriodora*, *Cymbopogon winterianus*, *Lippia alba* and *Ocimum americanum* essential oils (O). Mean values ± standard deviation.

O	D	*Colletotrichum gloesporioides*					*Botrytis cinerea*					*Monilinia fructicola*				
		MG*	ICP	μ	Ω	EC_{50}	MG	ICP	μ	Ω	EC_{50}	MG	ICP	μ	Ω	EC_{50}
A. citriodora	0.0	6.23 ± 0.32d	2.23 ± 3.43d	1.45 ± 0.10c	1.2	0.41	7.47 ± 0.42c	2.63 ± 3.18c	2.43 ± 0.24d	0.8	0.63	7.70 ± 1.02c	6.50 ± 7.96c	2.13 ± 0.18c	0.4	0.21
	0.2	3.51 ± 0.29c	43.64 ± 4.47c	0.71 ± 0.11c			7.06 ± 0.98bc	7.49 ± 11.76bc	1.87 ± 0.31c			5.03 ± 0.89b	37.61 ± 6.90b	1.35 ± 0.16b		
	0.4	3.23 ± 1.0c	48.13 ± 16.09c	0.62 ± 0.15c			6.99 ± 0.87bc	8.80 ± 8.76bc	1.31 ± 0.57b			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a		
	0.6	1.20 ± 1.05b	80.73 ± 16.83b	0.16 ± 0.19b			6.48 ± 0.98b	13.95 ± 12.30b	1.04 ± 0.56b			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a		
	0.8	0.94 ± 1.03ab	84.89 ± 17.04ab	0.14 ± 0.24b			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a		
	1.0	0.80 ± 1.05ab	87.16 ± 17.04ab	0.10 ± 0.14a			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a		
	1.2	0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a		
	1.4	0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a		
C. winterianus	0.0	6.23 ± 0.32e	2.10 ± 3.48e	1.53 ± 0.10f	1.2	0.86	8.08 ± 0.46c	2.15 ± 4.06c	3.02 ± 0.16d	1.0	0.52	7.70 ± 1.02c	6.50 ± 7.90c	2.13 ± 0.18c	0.4	0.23
	0.2	5.55 ± 0.96de	12.32 ± 14.23de	1.36 ± 0.15ef			7.73 ± 0.76c	6.12 ± 6.89c	2.43 ± 0.25c			6.05 ± 0.69b	21.41 ± 8.74b	1.53 ± 0.18b		
	0.4	5.01 ± 0.51d	19.57 ± 8.25cd	1.26 ± 0.11de			6.83 ± 1.74c	16.67 ± 20.52c	1.97 ± 0.46c			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a		
	0.6	3.40 ± 0.66b	45.39 ± 10.72b	0.67 ± 0.17b			1.31 ± 1.36b	83.74 ± 16.94ab	0.63 ± 0.71b			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a		
	0.8	4.28 ± 0.53c	31.26 ± 8.56c	0.99 ± 0.10c			1.88 ± 1.92a	76.69 ± 23.86b	0.40 ± 0.41ab			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a		
	1.0	4.47 ± 0.84c	28.34 ± 13.52c	1.09 ± 0.11cd			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a		
	1.2	0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a		
	1.4	0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a		
L. alba	0.0	6.23 ± 0.32e	2.23 ± 3.43d	1.53 ± 0.10d	1.2	0.76	7.93 ± 0.28f	1.38 ± 2.66f	2.54 ± 0.15d	1.0	0.64	8.11 ± 0.49c	4.15 ± 4.98c	2.21 ± 0.11c	1.2	0.41
	0.2	4.77 ± 1.13d	23.48 ± 18.46c	1.14 ± 0.20c			7.4 ± 0.24e	6.55 ± 3.08e	2.03 ± 0.45c			–	–	–		
	0.4	4.90 ± 1.78cd	24.74 ± 24.97c	1.10 ± 0.26c			6.23 ± 0.28d	21.39 ± 3.65d	1.35 ± 0.32b			2.56 ± 0.78b	68.42 ± 9.72b	0.41 ± 0.14b		
	0.6	3.38 ± 0.50bcd	45.77 ± 8.13b	0.84 ± 0.11b			3.93 ± 0.30c	50.39 ± 3.82c	0.94 ± 0.25b			–	–	–		
	0.8	3.73 ± 0.40bc	40.20 ± 6.56bc	0.77 ± 0.10b			4.57 ± 0.39b	42.33 ± 5.03b	0.97 ± 0.43b			1.10 ± 1.15a	86.36 ± 14.21a	0.16 ± 0.17a		
	1.0	3.52 ± 0.86b	43.43 ± 13.89b	0.71 ± 0.21b			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a			–	–	–		
	1.2	0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a		
	1.4	0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a							
O. americanum	0.0	6.23 ± 0.32b	2.23 ± 3.43b	1.53 ± 0.10b	1.2	0.64	8.24 ± 0.17g	0.82 ± 0.95g	2.59 ± 0.12e	1.4	0.74	7.70 ± 1.02d	6.50 ± 7.96d	2.13 ± 0.18d	0.8	0.41
	0.2	5.23 ± 1.47b	19.61 ± 19.62b	1.39 ± 0.24b			6.87 ± 0.64f	16.54 ± 7.87f	1.93 ± 0.20d			4.94 ± 0.33c	35.74 ± 4.31c	1.03 ± 0.09c		
	0.4	5.50 ± 1.33b	15.59 ± 17.56b	1.36 ± 0.27b			5.69 ± 0.47e	30.93 ± 5.74e	1.33 ± 0.30c			4.03 ± 0.54cb	47.57 ± 7.11b	0.76 ± 0.08b		
	0.6	5.65 ± 1.14b	11.18 ± 16.78b	1.40 ± 0.18b			4.74 ± 0.34d	42.48 ± 4.71d	1.27 ± 0.29c			3.52 ± 0.14b	54.21 ± 1.94b	0.622 ± 0.01b		
	0.8	0.89 ± 1.40a	85.58 ± 22.56a	0.13 ± 0.23a			4.78 ± 0.31d	41.89 ± 3.83d	1.21 ± 0.34c			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a		
	1.0	0.85 ± 1.15a	86.31 ± 18.44a	0.10 ± 0.14a			3.76 ± 0.58c	54.28 ± 7.08c	0.66 ± 0.29b			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a		
	1.2	0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a			1.03 ± 0.94b	87.40 ± 11.80b	0.33 ± 0.33ab			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a		
	1.4	0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a			0.00 ± 0.00a	100 ± 0.00a	0.00 ± 0.00a		

* Equal letters for the variables do not differ by the Tukey test at 5% probability of error.

Table 3
Germination of *Monilinia fructicola* spores submitted to doses (D, mL/L) of *A. citriodora*, *C. winterianus*, *L. alba* and *O. americanum* essential oils. Mean values ± standard deviation.

D	Essential oils*			
	A. citriodora	*C. winterianus*	*L. alba*	*O. americanum*
0.0	74.99 ± 23.61 a	100.00 ± 0.00 a	72.85 ± 14.21 a	68.51 ± 4.57 a
0.2	0.00 ± 0.00 b	2.64 ± 0.16 b	16.39 ± 5.94 b	6.63 ± 2.67 b
0.4	0.00 ± 0.00 b	0.00 ± 0.00 b	10.99 ± 16.91 b	6.51 ± 0.93 b
0.6	0.00 ± 0.00 b	0.00 ± 0.00 b	4.21 ± 1.22 b	5.68 ± 0.76 b
0.8	0.00 ± 0.00 b	0.00 ± 0.00 b	4.09 ± 1.63 b	5.32 ± 2.01 b
1.0	0.00 ± 0.00 b	0.00 ± 0.00 b	2.81 ± 3.15 b	4.96 ± 1.32 b
1.2	0.00 ± 0.00 b	0.00 ± 0.00 b	2.05 ± 1.64 b	4.66 ± 2.55 b

* Equal letters for the essential oil do not differ by the Tukey test at 5% probability of error.

Table 4
Incidence (IN, %), spore production (SP, spore mL^{-1}) and control efficiency (CE, %), severity (S, %) and lesion diameter (LD, cm) in strawberries with artificial inoculation (AI) and no artificial inoculation (NAI) of *Colletotrichum* sp. when submitted to post-harvest treatments with essential oils. Mean values ± standard deviation.

Post-harvest treatment*	IN		SP	
	AI	NAI	AI	NAI
Control	100.00 ± 0.00 aA	75.00 ± 43.30 aB	1170.93 ± 1720.27 aA	36.25 ± 27.18 aB
A. citriodora	0.00 ± 0.00 cA	0.00 ± 0.00 cA	0.00 ± 0.00 cA	0.00 ± 0.00 aA
C. winterianus	100.00 ± 0.00 aA	75.00 ± 43.30 aB	751.75 ± 82.96 abA	46.56 ± 172.74 aB
L. alba	62.50 ± 48.41 bA	56.25 ± 49.60 abA	183.43 ± 251.51 bcA	9.37 ± 20.83 aA
O. americanum	87.50 ± 33.07 abA	25.00 ± 43.30 bcB	396.56 ± 522.38 bcA	0.62 ± 2.42 aA

Post-harvest treatment	S		LD		CE	
	AI	NAI	AI	NAI	AI	NAI
Control	7.75 ± 4.46 bc	1.12 ± 0.56 ab	0	0		
A. citriodora	0.00 ± 0.00 c	0.00 ± 0.00 c	100,00 ± 0.00 aA	100,00 ± 0.00 aA		
C. winterianus	21.56 ± 7.94 a	1.57 ± 0.81 a	10,04 ± 15.31 cA	28,64 ± 43.49 bA		
L. alba	11.8 ± 3.63 b	0.92 ± 0.89 b	46,97 ± 42.84 bA	44,58 ± 48.91 bA		
O. americanum	5.56 ± 1.92 bc	0.73 ± 0.75 b	32,59 ± 35.60 bcB	80,06 ± 38.73 aA		

* Equal capital letters in the row and lower-case letters in the column do not differ by the Tukey test at 5% probability of error.

There was incidence of *M. fructicola* in all performed treatments on both artificially inoculated peaches and on fruits without artificial inoculation, demonstrating the presence of latent infections in the fruits. There was 100% incidence of brown rot for the control, with no statistical difference in the form of inoculation. However, peaches with artificial inoculation showed a higher incidence for the other treatments than fruits without artificial inoculation, with 100% (Table 6).

For peaches which were not artificially inoculated, it was possible to verify differences between post-harvest treatments with the *A. citriodora* and *C. winterianus* essential oils significantly reducing the incidence of rot with 40 and 50%, respectively (Table 6).

The post-harvest treatments did not influence the severity percentage, lesion diameter (LD) or the control efficiency (CE) for peaches with artificial inoculation. However, all treatments with essential oils reduced the severity of brown rot for fruits without artificial inoculation, differing statistically from the control. There was a 64% reduction in brown rot severity for the *A. citriodora* essential oil, 63% for the *C. winterianus* essential oil, 55.7% for the *O. americanum* essential oil and 49% for the *L. alba* essential oil (Table 6).

Table 5
Incidence (IN, %), severity (S, %), spore production (SP, spore mL^{-1}), lesion diameter (LD, cm) and control efficiency (CE, %) in peaches with artificial inoculation (AI) and no artificial inoculation (NAI) of *Botrytis cinerea* when subjected to post-harvest treatments. Mean values ± standard deviation.

Post-harvest treatment*	IN		S		SP	
	AI	NAI	AI	NAI	AI	NAI
Control	94.00 ± 24.20 aA	19.00 ± 39.03 aB	9.00 ± 4.66 bA	2.00 ± 4.24 aB	264.37 ± 210.21 aA	52.18 ± 139.84 aB
A. citriodora	87.00 ± 33.07 abA	0.00 ± 0.00 aB	20.00 ± 15.34 aA	0.00 ± 0.00 aB	159.68 ± 255.08 abA	0.00 ± 0.00 aB
B. winterianus	31.00 ± 46.35 cA	0.00 ± 0.00 aB	1.00 ± 1.36 cA	0.00 ± 0.00 aA	7.81 ± 13.57 cA	0.00 ± 0.00 aA
L. alba	100.00 ± 0.00 aA	25.00 ± 43.30 aB	15.00 ± 8.46 abA	4.00 ± 8.52 aB	75.31 ± 35.24 bcA	11.87 ± 24.23 aA
O. americanum	56.00 ± 49.60 bcA	0.00 ± 0.00 aB	2.00 ± 2.52 cA	0.00 ± 0.00 aA	93.43 ± 104.81 bcA	0.00 ± 0.00 aB

Post-harvest treatment	LD		CE	
	AI	NAI	AI	NAI
Control	1.35 ± 0.50 aA	0.29 ± 0.63 aB	0.00 ± 0.00	0.00 ± 0.00
A. citriodora	1.76 ± 0.28 aA	0.00 ± 0.00 aB	7.00 ± 24.05 bB	100.00 ± 0.00 aA
C. winterianus	0.14 ± 0.23 bA	0.00 ± 0.00 aA	89.00 ± 17.21 aA	100.00 ± 0.00 aA
L. alba	1.76 ± 0.61 aA	0.42 ± 0.74 aB	0.50 ± 1.64 bB	75.00 ± 43.30 bA
O. americanum	0.21 ± 0.33 bA	0.00 ± 0.00 aA	84.00 ± 24.61 aA	100.00 ± 0.00 aA

* Equal capital letters in the row and lower-case letters in the column do not differ by the Tukey test at 5% probability of error.

Spore production was higher for most treatments of fruits with artificial inoculation. However, the form of inoculation for *L. alba* essential oil did not influence spore production. The lowest spore production was found in this oil, with 1397 spores/mL, only presenting statistical equality with the *C. winterianus* essential oil with 3011.5 spores/mL, and statistically differing from the other treatments (Table 6).

All treatments with essential oil reduced the lesion diameter for peaches without inoculation, with the *C. winterianus*, *A. citriodora* and *O. americanum* essential oils showing the lowest averages, with 2.20, 2.48 and 2.51 cm, respectively, statistically differing from the control.

The control efficiency variable (CE) was similar to the results achieved in the lesion diameter. All treatments for fruits with no artificial inoculation (NAI) provided high control efficiency, with 64.42% for the *A. citriodora* essential oil, 62.11% for the *C. winterianus* essential oil, 55.61% for the *O. americanum* essential oil and 45.43% for the *L. alba* essential oil, statistically differing from the control (Table 6).

The post-harvest treatments carried out with peaches showed differences between them for the (α) variable, with the *A. citriodora* essential oil drastically reducing the progress of the disease with 72.6, and only statistically differing from the control with an area of approximately 110. It was possible to verify differences between the inoculation process for the (α) variable, with inoculated fruits (AI) presenting a larger area than the fruits not artificially inoculated (NAI)

Table 6
Incidence (IN, %), severity (S, %), lesion diameter (LD, cm), spore production (SP, spore mL^{-1}) and control efficiency (CE, %) in peach, Precocinho cultivar, with artificial inoculation (AI) or no artificial inoculation (NAI) of *Monilinia fructicola* submitted to post-harvest treatments. Mean values ± standard deviation.

Post-harvest treatment*	IN		S	
	AI	NAI	AI	NAI
Control	100.00 ± 0.00 aA	100.00 ± 0.00 aA	100.00 ± 0.00 aA	100.00 ± 0.00 aA
A. citriodora	100.00 ± 0.00 aA	40.00 ± 48.98 bB	93.23 ± 10.36 aA	35.92 ± 45.38 bB
C. winterianus	100.00 ± 0.00 aA	50.00 ± 50.02 bB	98.54 ± 2.73 aA	36.99 ± 40.38 bB
L. alba	100.00 ± 0.00 aA	70.00 ± 45.82 abB	99.57 ± 1.06 aA	50.92 ± 37.07 bB
O. americanum	100.00 ± 0.00 aA	60.00 ± 48.98 abB	99.34 ± 1.96 aA	44.25 ± 44.73 bB

Post-harvest treatment	SP		CE	
	AI	NAI	AI	NAI
Control	20,504.00 ± 3661.59 aA	10,228.00 ± 4884.05 aB	0.00 ± 0.00	0.00 ± 0.00
A. citriodora	7161.05 ± 4802.49 bA	818.50 ± 1650.40 bB	5.04 ± 10.09 aB	64.42 ± 45.22 aA
C. winterianus	3011.50 ± 1800.40 cdA	82.5 ± 87.04 bB	0.76 ± 1.75 aB	62.11 ± 40.92 aA
L. alba	1397.00 ± 810.67 dA	134.00 ± 152.27 bA	0.71 ± 1.16 aB	45.43 ± 39.33 aA
O. americanum	6488.00 ± 1749.65 bcA	229.00 ± 340.28 bB	1.11 ± 2.47 aB	55.61 ± 44.91 aA

* Equal capital letters in the row and lower-case letters in the column do not differ by the Tukey test at 5% probability of error.

(Fig. 2).

4. Discussion

The growth inhibitory dose of *Colletotrichum* sp. with 1.2 mL/L for all essential oils is in accordance with the literature. Positive results for the control of *Colletotrichum* sp. have already been observed using essential oils such as *Aloysia citriodora*, *Cymbopogon citratus*, *Psidium guajava*, *Eucalyptus citriodora*, *Eucalyptus staigeriana*, *Mentha arvensis*, *Artemisia dracunculus*, *Astrocaryum murumuru*, *Mauritia flexuosa*, *Schinus terebinthifolius*, and *Lippia menosides* (Aquino et al., 2014; Carnelossi et al., 2009; Ferreira et al., 2012; Oliveira Junior et al., 2013; Oliveira et al., 2019a,b; Pereira et al., 2007; Souza Júnior et al., 2009).

The positive results verified in both *in vitro* and post-harvest experiments demonstrate the high potential of the *Aloysia citriodora* essential oil as a "sustainable fungicide" (renewable source) emerging as an alternative, as it reached 100% efficiency in the control of *Colletotrichum* sp.

There are no data in the literature which prove the efficiency of essential oils tested in the control of *Botrytis cinerea*, and the data obtained in this experiment are innovative. The *C. winterianus* essential oil stood out from the others, showing high efficiency of disease control with values of 90 to 100% in post-harvest treatment. The *O. americanum* essential oil also showed satisfactory control of *Botrytis cinerea* with control efficiency of 85 and 100% in post-harvest.

Positive results have already been found in the control of *Botrytis cinerea* with other essential oils (Aguilar-González et al., 2015; Combrinck et al., 2011; Lorenzetti et al., 2011; Salgado et al., 2003).

The *Aloysia citriodora* and *C. winterianus* essential oils stood out in the control of *Monilinia fructicola*, as they inhibited mycelial growth and spore germination in low concentrations. According to Pansera et al. (2015), the *Cymbopogon camphora* and *Eucalyptus globulus* essential oils are also effective in controlling mycelial growth, however they require higher doses than the doses tested for the oils in our experiment.

The post-harvest treatment carried out with the *Aloysia citriodora* essential oil at a dose of 0.4 mL/L stood out from the others, promoting values above 60% of disease control in the analyzed variables. The results corroborate Pansera et al. (2015), who verified the efficiency of *C. camphora* essential oil in reducing the incidence and the lesion diameter for *M. fructicola* in *Chimarrita* cv. peaches, and similar efficiency was verified with the fungicide Orthocide (100% control).

Some authors cite the potential of *Cymbopogon citratus* essential oil (of the same genus as *Cymbopogon winterianus*) in the control of *Colletotrichum gloesporioides* in post-harvest fruit, such as in reducing the lesion diameter in passion fruit (Aquino et al., 2014), in reducing the area under the disease progress severity curve (α), in the severity of the disease in papaya fruits (Carnelossi et al., 2009) and in reducing the incidence and lesion diameter in peaches (Pansera et al., 2015).

The high levels of disease found in the post-harvest for the *C. winterianus* essential oil may be linked to the possible phytotoxic effect promoted by the treatment. The possible phytotoxic action was also

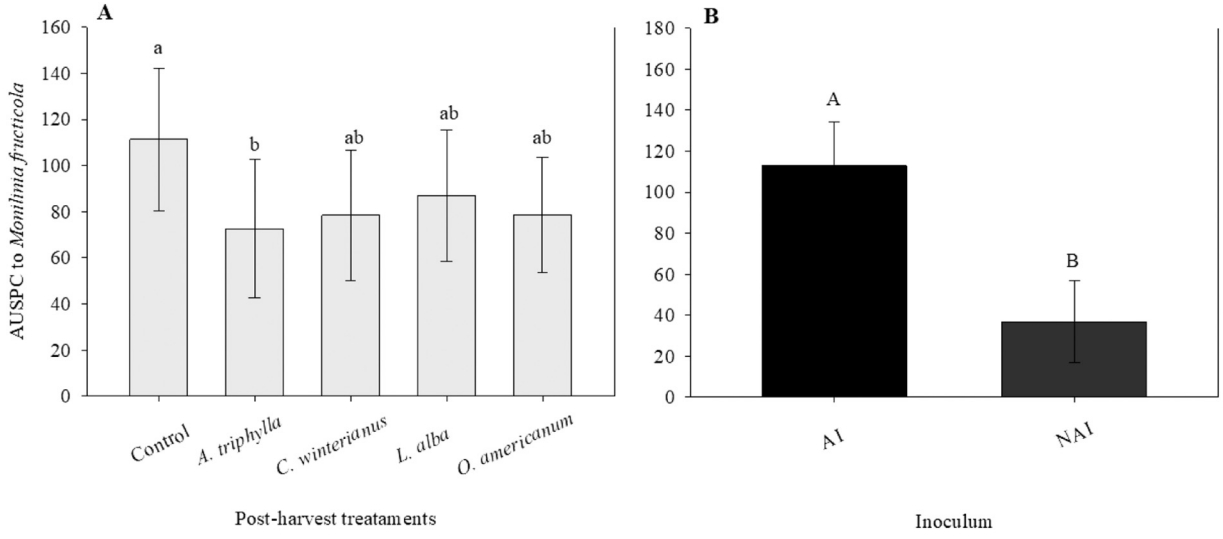
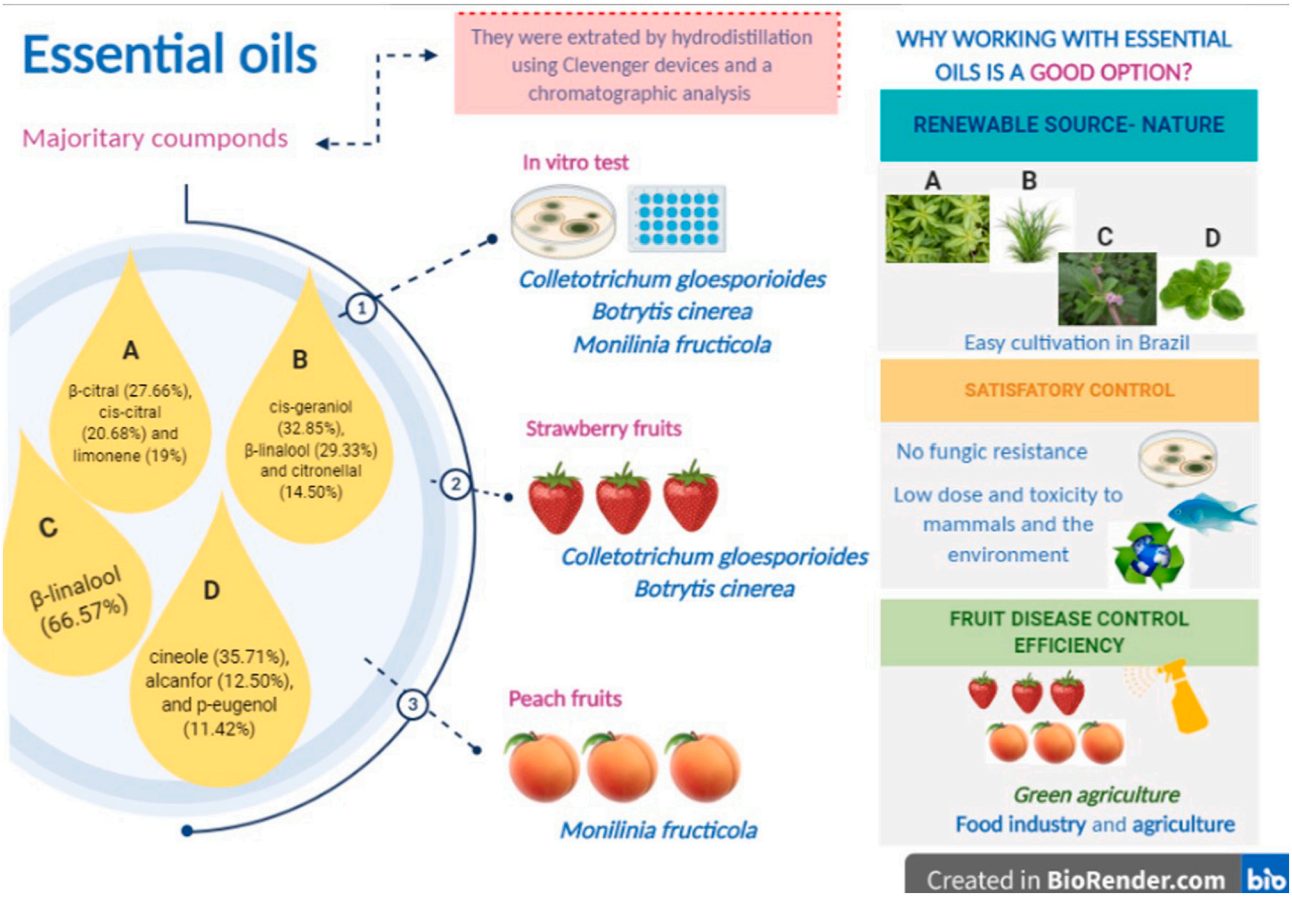


Fig. 2. Area under the severity progress curve (AUSPC - α) of *Monilinia fructicola* in peaches submitted to post-harvest treatments with Control, and *A. citriodora*, *C. winterianus*, *L. alba* and *O. americanum* (A) essential oils with artificial inoculation (AI) and without artificial inoculation (NAI) (B).
*Equal letters for the variables do not differ by the Tukey test at 5% probability of error. Bars represent mean standard deviation of treatments.



Scheme 1. The scheme shows the main experiments carried out at work, as well as some important points for dissemination and about the potential use of essential oils in the maintenance of post-harvest fruits and in agriculture. Created with BioRender.com.

suggested by Lorenzetti et al. (2011) for another essential oil when testing the citrus oil in the post-harvest of strawberries. They also found the highest values of gray mold incidence which altered the coloring of the fruits, abnormally yellowing them and facilitating infection by the pathogen.

The presence of the disease in fruits without artificial inoculation demonstrates the presence of latent infections inside the fruit which develops when there are ideal conditions. The same was found in a study by Cia et al. (2009), in which grape berries inoculated with *Colletotrichum gloesporioides* had a higher disease incidence percentage than naturally infected berries, artificially inoculated fruits develop their latent infections, in addition to the amount of inoculum inserted in the fruit after the wound.

The positive results in the *in vitro* experiment were not always repeated in the post-harvest experiment, and that is why it is important to adapt essential oils with their different compositions, their doses and their effects on the physiology of the fruits, because in addition to controlling pathogens, it becomes necessary if they do not change qualitative aspects for commercialization.

Aromatic plants have terpenes and flavonoids in their composition which are endowed with antimicrobial activity and act in the chemical defense of plants against fungi and bacteria (Castro et al., 2001). The control of microorganisms with essential oils is justified by two premises, namely their chemical compounds being able to act directly on the fungus, and/or induce the fruit to produce enzymes, proteins and defense substances (Yang et al., 2012).

Recent research suggests that the resistance-inducing mechanism is involved in the activation of enzymes, accumulation of antifungal compounds, proteins, increase in reactive oxygen species and lignification of epidermal cells (Vilanova et al., 2014). Therefore, Freddo et al. (2016) found that the application of the *Aloysia citriodora* essential oil in cucumber seedlings provided an enzymatic increase in β-1,3-glucanases, phenylamine ammonia lyase (PAL), chitinases and other proteins related to the defense of the host. Previous studies have suggested that the induction of β-1,3-glucanase enzymes is correlated with increased resistance to *Botrytis cinerea*, providing a significant reduction in the degradation of strawberries (Pombo et al., 2011).

The *Cymbopogon citratus* essential oil has already been proven to alter the metabolism of the vine, causing an increase in the chitinase enzyme activity (Maia et al., 2014). The antimicrobial activity of chitinase is based on its ability to hydrolyze chitin polymers, weakening the cell wall and making microbial cells osmotically sensitive (Selitrennikoff, 2001).

When Ben-Jabeur et al. (2015) tested *Thymus capitatus* essential oil to control *Botrytis cinerea*, they found a reduction in the incidence of the disease, as well as an increase in peroxidases and phenolic compounds. According to Singh et al. (2006), the accumulation of peroxidases is the first sign of activating the plant's defense, causing oxidative explosion by an accumulation of reactive oxygen species (ROS). ROS have several effects on the defense responses of the plant, including the activation of defense genes and compounds (Levine et al., 1994).

The direct-action mechanism of essential oils is associated with the lipophilic character of oil compounds which strongly bind to the membrane, changing selectivity and facilitating penetration into membranes, causing loss of energy by microbial cells (Knaak and Fiuza, 2010; Lambert et al., 2001). Direct inhibition of fungal growth by essential oils often involves preventing growth and sporulation of hyphae, disrupting nutrient absorption and metabolism, disrupting the plasma membrane, disrupting the mitochondrial structure, and interfering with enzymatic and respiratory reactions (Patel and Jasrai, 2011). Changes in morphology and surface damage of fungal structures have also been

observed (Oliveira et al., 2019a,b; Sharma et al., 2017).

Oliveira et al. (2019a,b) observed structural changes on the surface of *Colletotrichum acutatum* when subjected to *Lippia sidoides* oil through scanning electron microscopy techniques, such as superficial wrinkles in the fungus hyphae, in addition to flaking, distortion and their destruction, making them unfeasible. In another study, Oliveira et al. (2019a,b) observed cellular changes in *Rhizopus stolonifer* subjected to the same essential oil such as reduced cellular regularity, disorganized organelles, loss of cytoplasm and plasma membrane with low functionality.

The antimicrobial and resistance-inducing activity present in essential oils can be justified through their chemical composition. For example, the citral compound (present in the *Aloysia citriodora* essential oil) showed control of fungi such as *Penicillium* spp. and *Geotrichum candidum* when evaluated in the post-harvest of citrus fruits (Klieber et al., 2002). These authors checked citral CMI with 3 ml/L *in vitro*. Paulus et al. (2013) also cite citral and limonene as probable fungitoxic compounds, proving their high antifungal effect *in vitro* and post-harvest against *Colletotrichum* sp. in the present work.

Geraniol, present in *C. winterianus* essential oil, is frequently reported due to its high antimicrobial activity (Duarte et al., 2007; Jirovetz et al., 2007), as well as citronellal and citronellol (Kordali et al., 2007). *L. alba* essential oil has β-Linalool (66.57%) in its composition. This compound, together with citral, geraniol and citronellol, has high antifungal activity against *Fusarium* sp. (Necha and Barrera, 2008).

The eugenol compound present in the *O. americanum* essential oil also has a high fungicidal potential (Combrinck et al., 2011). When tested in the post-harvest of apples, it reduced the incidence of pathogens such as *Phlyctema vagabunda*, *Penicillium expansum*, *Botrytis cinerea* and *Monilinia fructigena* by 90% (Amiri et al., 2008).

However, the efficiency of these major components may have less activity in relation to the complete essential oil, demonstrating the importance of the synergistic effect of trace elements (Mourey and Canillac, 2002).

Finding viable solutions to control fruit diseases has become important due to the constant mutation of microorganisms, the resistance of fungal isolates to already-tested chemicals, and the search for contaminant-free foods. Thus, the efficiency of the essential oils tested in this work can modify the scenario of natural fungicides. Moreover, most essential oils are classified by the FDA (Food and Drug Administration) as GRAS (Generally Recognized as Safe), so there is a growing interest in using them to treat fruits and vegetables (González-Aguilar et al., 2008).

Based on these observations and the innovative results presented in this experiment, it is believed that essential oils have great potential for both generating products in the food industry and for the chemical pesticides industry, as they present high efficiency in controlling diseases in strawberries and peaches. In addition, they are aromatic plants which are easy to grow, producing essential oil that is highly safe for consumers and the environment (Scheme 1).

5. Conclusion

Aloysia citriodora, *C. winterianus*, *Lippia alba* and *Ocimum americanum* essential oils are promising for the control of diseases in strawberries and peaches, showing potential in promoting sustainable agriculture.

Declaration of competing interest

The authors declare that there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2020.108980.

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